FURTHER STUDIES ON THE NATURE OF THE PHENOMENON OF LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES: TOXIC FACTORS DERIVED FROM THE BLOOD SERUM

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As reported previously (1) the *sine qua non* of the phenomenon of local skin reactivity to bacterial filtrates is that the second injection be given *via* the blood stream. Whilst large doses reinjected locally remain ineffective, an amazingly small quantity of toxic filtrate may elicit severe hemorrhagic necrosis at the prepared skin site (*i.e.*, with some batches of meningococcus filtrates less than 0.000009 cc., per kilo of body weight).

Inasmuch as these observations suggest that there occurs some interaction between the toxic substances injected and the blood prior to the elicitation of the reaction, it was of interest to determine whether a disturbance in the colloidal state of the blood would bring about formation of reacting factors. The results of these studies are embodied in the present paper.

EXPERIMENTAL

I

Testing of Sera for Skin-Preparatory Factors.—The abdominal skin of rabbits was prepared by single or four simultaneous injections of sera tested. A dose of 0.25 cc. was used for each intradermal injection. 24 hours later the rabbits received each a single intravenous injection of *B. typhosus* culture filtrate, in a dose of 2 cc., per kilo of body weight. Reactions were read 4 to 5 hours later. Rabbits succumbing within 4 hours after the intravenous injection were not recorded. The *B. typhosus* culture filtrates were made according to methods previously described (2). The results are summarized in Table I.

As is seen from Table I, one batch of crude commercial antityphoid horse serum and several batches of chemically concentrated immune

sera showed skin-preparatory potency. However, the antityphoid horse serum was stored in these laboratories for 2 years and, although it was found sterile at the time of the tests, it might have been con-

TABLE I

Sera Tested for Skin-Preparatory Factors

Sera injected intradermally	Total No. of areas tested	No. of negative reactions	No. of doubtful reactions	No. of positive reactions
Comm. antityphoid horse serum	12	0	0	12
3 antityphoid rabbit sera* 90, 78, 59	32	32	0	0
2 batches of pooled normal rabbit sera	36	36	0	0
Pooled sera of rabbits injected with typhoid fltr. kwt. immediately before bl.	4	0	0	4
Pooled sera of rabbits injected with typhoid fltr. 3 cc. kwt. 24 hrs. before bl.	16	16	0	0
Serum of rabbit injected with *coli* fltr. 3 cc. kwt. 4 hrs. before bl.	12	12	0	0
Serum of rabbit injected with typhoid fltr. 3 cc. kwt. 3 hrs. before bl.	12	12	0	0
Serum of rabbit injected with typhoid fltr. 2 cc. kwt. twice 24 hr. interval; bleeding 2 hrs. after 2nd injection	16	16	0	0
7 antityphoid goat sera*	28	27	1	0
Mount Sinai antityphoid sera of Horses* 2, 3, 4, 5 and 6	20	20	0	0
B. H. typhoid diagnostic serum	16	16	0	0
2 batches anti-*shigae* rabbit serum	24	24	0	0
2nd wk. typhoid pt. serum	12	12	0	0
23 batches comm. antimeningococcus horse serum	180	178	2	0
2 diagnostic B. H. $Pneumococcus_3$ sera	20	20	0	0
B. H. therapeutic $Pneumococcus_2$ Serum 930	16	16	0	0
B. H. therapeutic $Pneumococcus_2$ Serum 28/934	20	0	0	20
B. H. therapeutic $Pneumococcus_3$ serum	20	4	0	16
Comm. antimening. conc. serum	8	4	0	4
Mount Sinai antityphoid conc. horse serum	16	0	00	16

kwt. = per kilo of body weight. B.H. = New York Board of Health. bl. = bleeding. fltr. = filtrate. pt. = patient. comm. = commercial. conc. = concentrated.

* Bleedings obtained at various stages of immunization were selected for these tests.

taminated sometime during its prolonged storage and resterilized by the preservative. It is also clear that chemically concentrated sera are grossly contaminated during the treatment. As a matter of fact, the Mount Sinai antityphoid concentrated serum prepared in these laboratories (3) when cultured a number of times during the process of concentration proved consistently to be heavily contaminated by a variety of microorganisms (*i.e.*, *B. proteus*, *coli*, *subtilis*, staphylococcus, etc.) prior to the routine addition of 0.6 per cent tricresol and ether mixture. The majority of these bacteria are capable of producing toxic substances of high skin-preparatory potency (4). Moreover, the rabbit serum obtained by heart puncture immediately after intravenous injection of as much as 4 cc. of *B. typhosus* culture filtrate, per kilo of body weight, also possessed a definite skin-preparatory potency. On the other hand, no skin-preparatory factors were found in a great variety of normal sera, immune sera of various animals obtained during different stages of immunization and sera of rabbits injected with large doses of toxic filtrates some hours before bleeding, all of which were collected under sterile precautions and properly preserved.

Testing of Sera for Reacting Factors.—Rabbits were prepared by one intradermal injection of 0.25 cc. of a potent *B. typhosus* "agar washings" filtrate (2). 24 hours later they received each a single intravenous injection of the serum tested. The reactions were read 4 to 5 hours later. Each serum was tested in a group of three rabbits, unless stated otherwise.

The following sera tested in doses per kilo of body weight, indicated below, were found totally devoid of reacting potency.

(1) Normal human serum, 1 cc.; (2) antimeningococcus horse Serum H_7Bl_{405}, 3 cc.; (3) antimeningococcus rabbit Serum S_{10}, 1 cc.; (4) antimeningococcus rabbit Serum S_{215}, 1 cc.; (5) antimeningococcus horse Serum H_7Bl_{225}, 2 cc.; (6) normal rabbit serum, 2 cc.; (7) normal guinea pig serum, 2 cc.; (8) normal rat serum, 2.5 cc. of 1:10 dilution; (9) normal chicken serum, 1 cc.; (10) antityphoid immune chicken serum, 1 cc.; and (11) pooled serum of rabbits injected intravenously with 2.5 cc. of *B. typhosus* culture filtrate, per kilo of body weight, 3 hours before bleeding, 5 cc. (tested in four rabbits).

The commercial antityphoid horse serum above referred to which

was shown to contain skin-preparatory factors was also tested for reacting potency in four rabbits in a dose of 6 cc., per kilo of body weight. There were obtained severe reactions in three rabbits.

Several rabbits were injected intravenously with 4 cc. of *B. typhosus* "agar washings" filtrate, per kilo of body weight, and bled immediately afterwards. The pooled sera showed high reacting potency.[1]

Testing of Chicken Plasma for Skin-Preparatory and Reacting Factors

The following batches were tested.

In tests for skin-preparatory factors, one intradermal injection of 0.25 cc. of undiluted plasma was given to each rabbit. The intravenous dose consisted of 200 *B. typhosus* reacting units, per kilo of body weight (5). In tests for reacting factors, the preparatory dose consisted of one intradermal injection of 0.25 cc. of the undiluted *B. typhosus* "agar washings" filtrate into each rabbit and of a single intravenous injection 24 hours later of 1 cc. per kilo of body weight, of plasma tested.

Normal plasma; plasma of chicken immunized intravenously with *Streptococcus viridans* culture filtrate for 5 weeks and obtained 24 hours after the last injection; plasma of chicken immunized intravenously with the above filtrate for 2 months and obtained 4 days after the last injection; and plasma of chicken immunized intravenously with large doses of *B. typhosus* "agar washings" filtrates for 5 weeks, obtained 24 hours after the last injection.

The results of these experiments, which represent readings of three surviving rabbits for each plasma tested, were entirely negative.

The experiments described thus far demonstrate clearly that the toxic substances (*i.e.* skin-preparatory and reacting factors) present in some contaminated sera and the sera of rabbits receiving large doses of bacteria intravenously immediately before bleeding, cannot be demonstrated in plasma and whole serum of various animals injected with large doses over protracted periods of time or a few hours before bleeding.

In the following part of the paper there are described experiments with precipitates and supernatant fluids obtained from mixtures of precipitinogen-containing sera with homologous precipitating antisera.

[1] Titrations to end-point suggested an increase in toxicity. Further studies are under way.

II

The precipitating antisera employed in this part of the work were as follows:

1. Anti-Horse Rabbit Sera.—These sera were usually prepared by a 4 week course of intravenous immunization with normal horse serum. Injections were made consecutively twice a week. The initial dose was 0.5 cc. and the last dose 18 cc. Trial bleedings were obtained on the 6th day after the last injection. When the precipitating titer was sufficiently high the rabbits were bled from the heart the same day or the day following the trial bleedings. Most sera when undiluted showed abundant precipitate in mixtures with horse serum diluted 1:100.

2. Anti-Human Horse Sera.—The horse was immunized with heated meningococcus cultures and meningococcus culture filtrates of strains grown on media containing human blood for a period of 1 year. In addition normal human serum had been injected for several months. The methods will be described later in detail. The sera showed abundant precipitate in mixtures with human serum diluted 1:100.

3. Anti-Human Rabbit Sera.—These sera were prepared similarly to anti-horse rabbit sera.

4. Anti-Horse Goat Sera.—These were prepared by intravenous biweekly injections of horse serum for a period of 3 months. The sera were of a weak precipitating titer but abundant precipitate was obtained in mixtures of these sera with horse serum diluted 1:10.

In the work about to be described mixtures of the precipitinogen-containing serum with precipitating antiserum were made in proportions yielding abundant precipitate, unless stated to the contrary. The mixtures were incubated in a water bath at 37.5°C. for 2 hours, kept in the refrigerator overnight and centrifuged at high speed until the supernatant fluid was clear. The supernatant fluid was removed and the sediment suspended in a volume of 0.85 per cent NaCl solution equal to one-fourth of the volume of the antibody-containing serum.

Testing of Precipitates and Supernatant Fluids for Skin-Preparatory Factors

Each rabbit was prepared by a single intradermal injection of 0.25 cc. of the preparation tested and 24 hours later injected intravenously with 50 *B. typhosus* reacting units. The preparations were precipitates and supernatant fluids derived from mixtures of antimeningococcus anti-human horse Serum H_7Bl_{405} and normal human serum. The mixtures were made in proportions of 0.9 cc. of Serum H_7Bl_{405} to each of the following amounts of normal human serum: 0.025, 0.05 and 0.075 cc. of dilution 1:16; 0.1 cc. of dilution 1:2, and 0.1 cc. of undiluted serum. The mixtures were further treated, as described above.

The results which represent readings of three surviving rabbits for each preparation tested, were entirely negative.

Testing of Precipitates for Reacting Factors

Each rabbit received a single intradermal injection of 0.25 cc. of undiluted *B. typhosus* "agar washings" filtrate and 24 hours later an intravenous injection of 1 cc. of the suspension of precipitate tested, per kilo of body weight. There were no deaths following these injections. The results are recorded in Table II. In

TABLE II

Reacting Potency of Various Sera Precipitates

Precipitinogen-containing serum	Precipitating antiserum	Proportions of precipitinogen-containing serum and precipitating antiserum in mixtures	Results with precipitates		
			Total No. rabbits	Positive rabbits	Negative rabbits
Normal horse serum	Anti-horse rabbit Serum R I	0.33 cc. + 0.66 cc.	3	3	0
" " "	Anti-horse rabbit Serum R II	0.33 " + 0.66 "	3	2	1
" " "	Anti-horse rabbit Serum R III	0.66 " + 0.33 "	3	2	1
Comm. antimeningococcus horse Serum M_{18}	Anti-horse rabbit Serum R_{387}	0.66 " + 0.33 "	3	3	0
Mount Sinai antityphoid horse Serum H_4Bl_{232}	" "	0.66 " + 0.33 "	3	2	1
Mount Sinai antityphoid horse Serum H_4Bl_{196}	Anti-horse goat Serum G_{165}	1 cc. + 1 cc. diluted 1:5	3	2	1
Mount Sinai anti-*coli* horse Serum $H_{60}Bl_{188}$	Anti-horse rabbit Serum R_{388}	0.66 cc. + 0.33 cc.	3	3	0

comm. = commercial.

this table, the expression "positive rabbit" means that there was obtained in the rabbit's prepared skin site a severe hemorrhagic and necrotic lesion 4 to 5 hours after the intravenous injection. The lesions, which were intense in the majority of positive rabbits, were characteristic of the phenomenon of local skin reactivity to bacterial filtrates (6). By "negative rabbit" is meant absence of skin reaction following the intravenous injection.

As is seen from Table II, suspensions of precipitates derived from mixtures of precipitinogen-containing serum with precipitating anti-

serum possess a high reacting potency. The active preparations can be obtained from combinations of some normal animal sera with homologous antisera, as well as from combinations of sera of animals immunized with bacterial antigens with antisera against normal sera of the same animal species.

Titration of Reacting Potency of Precipitates

In these experiments, the precipitates were derived from mixtures of antimeningococcus anti-human horse sera with normal human sera, in proportions of

TABLE III

Titration of Reacting Potency of Serum Precipitates

Date of preparation	Precipitinogen-containing serum	Precipitating antiserum	Dilution of precipitate suspension	Total No. of rabbits tested	No. of negative rabbits	No. of doubtful rabbits	No. of positive rabbits
May 8, 1931	Pooled normal human sera	Antimeningococcus anti-human Serum H_7Bl_{229}	Undiluted	3	1	0	2
" 8, 1931	" "	" "	1:10	3	1	0	2
" 8, 1931	" "	" "	1:50	3	2	0	1
" 8, 1931	" "	" "	1:100	3	3	0	0
June 20, 1931	Normal human serum	" "	Undiluted	3	2	1	0
" 20, 1931	" "	" "	1:5	3	3	0	0
July 23, 1931	" "	" "	Undiluted	3	2	0	1
" 23, 1931	" "	" "	1:5	3	3	0	0
" 29, 1931	" "	Antimeningococcus anti-human horse Serum H_7Bl_{388}	Undiluted	3	0	0	3
" 29, 1931	" "	" "	1:5	3	3	0	0

0.9 to 0.05 cc., respectively. The precipitates obtained in the manner described above gave highly turbid suspensions and had a tendency to settle on the bottom of the test-tube. The aggregates were easily broken up by shaking. The dilutions 1:5 and 1:10 of these suspensions were also quite turbid. In dilution 1:50 the turbidity was slight and in dilution 1:100 the fluid was clear. Various dilutions of the suspensions were tested for reacting potency in rabbits prepared with *B. typhosus* "agar washings" filtrate. The tests were made in the course of the week following the preparation of the suspensions. The results are reported in Table III.

As is seen from Table III, the reacting potency of a given precipitate suspension can be quantitatively measured. As in the experiments with the phenomenon of local skin reactivity to bacterial filtrates, there are also observed natural fluctuations in susceptibility of rabbits employed.

The titrations recorded demonstrate that precipitates derived at various times from mixtures of the same ingredients and proportions may differ in reacting potency.

It is also evident that the amount of precipitate present in a given suspension has no direct bearing on its reacting potency. Thus, a

TABLE IV

Reacting Potency of Preparations from Completely and Partially Precipitated Mixtures

Antimeningococcus anti-human horse Serum H_7Bl_{405}	Normal human serum	Amount of precipitate* obtained	Reacting potency of precipitate	Reacting potency of supernatant fluid
1 part undiluted	0.1 part undiluted	Medium**	+	+
1 " "	0.1 " diluted 1:2	Maximum	+	0
1 " "	0.1 " " 1:8	Medium	+	+
1 " "	0.1 " " 1:16	Minimum	+	+
1 " "	0.1 " " 1:20	None	0	0

+ = one to three rabbits of each group of three tested showing severe hemorrhagic necrosis. 0 = no reaction obtained in a group of three rabbits.

* The precipitate was suspended in a volume of 0.25 per cent NaCl solution equal to one-fourth of the volume of horse serum used.

** Prozone.

preparation of only slight turbidity (*i.e.* dilution 1:50 of preparation of May 8, 1931) was potent, whilst several highly turbid preparations (*i.e.* dilutions 1:5 of the remaining suspensions) were totally inactive.

Similar observations were obtained with titrations carried out in the following manner.

Suspensions of precipitates were diluted in 0.85 per cent NaCl solution to a turbidity standard (McFarland nephelometer) of 500 million bacteria per cc. The test dose was 2 cc., per kilo of body weight.

Preparation I.—The mixture consisted of one part of antimeningococcus anti-human horse Serum H_7Bl_{486} and one part of human serum diluted 1:4. A suspension of standard turbidity and dilution 1:5 elicited severe reactions in two out of three rabbits tested. Dilution 1:10 gave negative results.

Preparation II.—The mixture consisted of one part of antimeningococcus anti-human horse Serum H_7Bl_{527} and one part of human serum diluted 1:4. A suspension of the precipitate of standard turbidity and suspensions two and four times more concentrated gave no reactions in rabbits.

It seems, therefore, that the reacting potency of a serum precipitate is not due to the mechanical effect of an inert colloidal suspension in the blood stream, but to some toxic quality associated with the precipitate. The following experiments were done in order to test this assumption further.

Reacting Potency of Precipitates and Supernatant Fluids Derived from Completely and Partially Precipitated Mixtures

In these experiments each preparation was tested in a group of three rabbits. The results are summarized in Table IV.

As is seen from Table IV, mixtures of serum with antiserum in proportions giving maximum precipitation yielded active precipitates and supernatant fluids devoid of reacting potency. Where precipitations were incomplete (*i.e.* in proportions yielding minimum and medium amounts of precipitates) both precipitates and supernatant fluids were potent.

The supernatant fluids were clear at the time of injections; namely, the day following preparation. Those derived from completely precipitated mixtures remained clear for an indefinite length of time. The supernatant fluids derived from partially precipitated mixtures became cloudy after several days in the refrigerator. The latter potent preparations were recentrifuged 2 to 3 times in the course of the following week. When no more cloudiness appeared in the refrigerator they were found on retest totally devoid of reacting potency. It became evident that the potency of supernatant fluids was due to the presence of a precipitate grossly invisible at the time of the injection.

Thus, it can be concluded from the above experiments that the reacting potency of a given preparation does not depend either on the amount of precipitate obtained or on the size of the aggregate formed.

Reacting Potency of Precipitates and Supernatant Fluids Derived from Mixtures of Bacterial Filtrates with Immune Sera

When a *B. typhosus* "agar washings" culture filtrate is mixed with an immune antityphoid horse serum in necessary proportions, there

occurs complete neutralization of *B. typhosus* reacting factors (5). The mixtures usually form abundant precipitates. In the experiments reported here, various numbers of reacting factors were mixed with a constant amount of antityphoid horse serum. The mixtures made in the proportions indicated in Table V, precipitates and the supernatant fluids derived from these mixtures were all tested for reacting potency. The results are summarized in Table V.

As is seen from Table V, precipitates derived from mixtures of *B. typhosus* filtrates with homologous immune serum were devoid of

TABLE V

Reacting Potency of Precipitates and Supernatant Fluids from Mixtures of Bacterial Filtrates with Immune Sera

Mixtures of *B. typhosus* reacting factors and antityphoid horse Serum H_4Bl_{222}		Reacting potency of mixtures, per kilo of body weight	Reacting potency of precipitates, per kilo of body weight	Reacting potency of supernatant fluids, per kilo of body weight
Nos. of reacting units	Amount of H_4Bl_{222}			
	cc.			
300	1	0	0	0
400	1	0	0	0
750	1	0	0	0
1000	1	+	+	0
1500	1	+	+	0
2000	1	+	+	0
3000	1	+	+	+

+ = three rabbits tested showing severe hemorrhagic necrosis. 0 = no reactions obtained in a group of three rabbits.

reacting potency if a sufficient amount of serum was used to neutralize *B. typhosus* reacting factors. When an excess of these factors was employed the precipitates proved active. Inasmuch as the potency of the precipitates appeared only with the increase in the amount of *B. typhosus* reacting factors in the mixture, it became evident that these precipitates, in contrast to those derived from serum plus antiserum mixtures, possessed no independent reacting potency.

Incidentally, it is of interest that in partially neutralized mixtures the non-neutralized *B. typhosus* reacting factors were associated with the precipitates. Thus, the precipitates derived from mixtures of

1000 and more reacting units with 1 cc. of serum were potent, whilst the supernatant fluid derived from a mixture of 2000 reacting units with 1 cc. of serum was inactive.

Origin of Reacting Potency of Serum Precipitates

A series of experiments was performed in order to determine which

TABLE VI

Origin of Reacting Potency of Serum Precipitates

Group No.	Precipitinogen-containing serum	Precipitating antiserum	Ratio of precipitinogen-containing serum to precipitating serum	Results
1	B. H. antimeningococcus horse Serum 347/169	Anti-horse rabbit Serum R_{403}	0.66 cc. + 0.33 cc.	+
2	Mount Sinai antimeningococcus horse Serum H_8Bl_{251}	" "	0.66 " + 0.33 "	0
3	Normal human Serum I	Anti-human rabbit Serum R_{392}	1 cc. + 0.2 cc.	0
4	Normal human Serum II	" "	1 " + 0.2 "	0
5	Normal human Serum III	" "	1 " + 0.2 "	0
6	Normal human Serum I	Antimeningococcus anti-human horse Serum H_7Bl_{388}	1 " + 0.2 "	+
7	Normal human Serum II	" "	1 " + 0.2 "	+
8	Normal human Serum III	Antimeningococcus anti-human horse Serum H_7Bl_{405}	1 " + 0.2 "	+

B.H. = New York Board of Health. + = one to three rabbits of each group of three tested showing severe hemorrhagic necrosis. 0 = no reactions obtained in a group of three rabbits.

of the ingredients of serum plus antiserum mixtures was responsible for the reacting potency of the precipitates. The results are reported in Table VI.

As is seen from Table VI, mixture of one horse serum with anti-horse rabbit serum yielded a potent precipitate (Group 1) whilst another

horse serum mixed with the same anti-horse rabbit serum gave an inactive precipitate (Group 2). In these experiments, then, the reacting potency was imparted by the precipitinogen-containing serum. In contrast, several batches of normal human serum mixed with the same anti-human rabbit serum gave inactive precipitates (Groups 3, 4 and 5). However, anti-human antimeningococcus horse serum mixed with the same batches of normal human serum yielded potent precipitates. Here, the reacting potency was, apparently, imparted by the precipitin-containing antiserum. It can be concluded, therefore, that the reacting potency of serum precipitates may originate from either of the ingedients of serum plus antiserum mixtures.

III

Testing of Inert Colloidal Suspensions for Reacting Potency

(*a*) In these experiments each of the substances was tested in a group of six prepared rabbits. The dose was 1 cc., per kilo of body weight. The suspensions made in 0.85 per cent NaCl solution were as follows: 4 per cent charcoal, 2 per cent infusorial earth, 4 per cent Witte's peptone, 4 per cent silicic acid and 10 per cent gelatine. All the substances were devoid of reacting potency. According to Sickles (7) galactose, gelatine and India ink have no reacting potency.

(*b*) Sickles reported (7) that agar was able to elicit reactions in rabbits previously prepared with meningococcus toxin. These experiments were repeated in the following manner.

The agar was dissolved in 0.85 per cent NaCl solution and sterilized by autoclaving at 250°F. for 15 minutes.

Rabbits were prepared with *B. typhosus* "agar washings" filtrate, as in above experiments. The intravenous dose was 2 cc., per kilo of body weight, of agar suspension tested. The suspensions contained 0.8, 0.5, 0.3, 0.2, 0.15, 0.08 and 0.01 per cent of agar. The dose of 2 cc. of 0.2 per cent of agar was the smallest quantity which elicited reactions in about 50 per cent of rabbits tested. Smaller doses (*i.e.*, 0.08 and 0.05 per cent) gave doubtful reactions. No reactions were obtained with 0.01 per cent of agar.

Sickles (7) showed that agar had no skin-preparatory effect. This has been also corroborated.

DISCUSSION AND CONCLUSIONS

Early in the work it was noticed that the skin-preparatory potency of various bacterial filtrates did not parallel their reacting potency. Thus, the majority of *B. typhosus* culture filtrates contained between 40 to 60 skin-preparatory units (2) and between 500 to 700 reacting units per 1 cc. (5). On the other hand, some meningococcus culture filtrates contained only ten preparatory units and between 2000 to 3000 reacting units. Moreover, culture filtrates of some bacterial species (*i.e.* streptococcus) (8), agar (7) and serum precipitates, as shown in this paper, were found lacking skin-preparatory effect but possessing considerable reacting potency. In the latter instances, the phenomenon could be elicited when rabbits were prepared with some bacterial filtrate of high skin-preparatory potency. Gratia and Linz reported that vaccine virus was able to elicit a state of reactivity to *B. coli* culture filtrates injected intravenously (9). According to Klein (10) vaccine virus had no reacting potency for skin areas prepared with potent bacterial filtrates.

It appears from observations made thus far that skin-preparatory and reacting factors are substances independent of each other. For this reason, the elicitation of the state of reactivity and production of injury in the reactive tissue can be considered as two independent phases of the phenomenon under discussion.

The first phase, *i.e.* elicitation of the state of reactivity, was interpreted by the present author as a functional disturbance in the tissue cells bringing about a transient state of vulnerability. The state is characteristically induced by bacterial filtrates[2] or by infection and is not a mere manifestation of the process of inflammation according to our present understanding of it (1).

The data presented in this paper concern the second phase of the phenomenon; *i.e.*, production of injury *via* the blood stream in tissue of induced vulnerability.

As has been seen, precipitates derived from mixtures of serum precipitinogen with precipitating antiserum were able to elicit severe

[2] Debonera, Tzortzakis and Falchetti (11) claimed that paraffin oil also induced a state of reactivity. Their observation could not be corroborated in this laboratory.

injury in skin sites prepared with bacterial filtrates. The amount of precipitate present in a given suspension had no direct bearing on its reacting potency. Some abundant serum precipitates as well as precipitates derived from neutralized mixtures of *B. typhosus* culture filtrates with immune sera were found inactive. The reacting potency did not depend on the size of the aggregate formed inasmuch as clear supernatant fluids obtained by centrifugalization of partially precipitated serum plus antiserum mixtures were able to elicit severe reactions. With the exception of agar, inert colloidal suspensions thus far tested (*i.e.* charcoal, infusorial earth, peptone, silicic acid, gelatine, galactose and India ink) possessed no reacting potency. The evidence thus far accumulated, therefore, points to the fact that the reacting potency of serum precipitates is not due to the mechanical effect of colloidal particles in the blood stream but to some toxic factors liberated or formed in the serum through the colloidal disturbance induced by the process of precipitation.

As pointed out before (1) the *sine qua non* of the phenomenon of local skin reactivity to bacterial filtrates is that the second injection be given *via* the blood stream. It is conceivable that, as in the case of sera, bacterial filtrates have no direct reacting potency but that they induce a colloidal disturbance in the blood which brings about liberation or formation of reacting factors. It remains to determine whether the effect of agar is due to a similar mechanism.

The phenomena of local skin reactivity to bacterial filtrates and serum precipitates seem to be best defined at present as the production of injury in tissues during a transient state of vulnerability induced by bacterial filtrates. The injury is produced by means of toxic factors formed in the blood stream through a colloidal disturbance in the latter.

In connection with the above described formation of reacting factors through a colloidal disturbance in blood serum, observations of Rous and his coworkers made in 1919 and 1920 are highly interesting. In order to remove the shock-producing property of a precipitating serum, they attempted repeated absorptions of hemolysins, hemagglutinins and precipitins. The absorbed serum, however, was able to engender shock. On the basis of these observations, they concluded that "there remains the interesting possibility of the presence in the serum

of a hitherto unrecognized toxic antibody. Further work alone can justify any speculation in this direction" (12).

As reported in this paper, sera concentrated by chemical means contained a considerable amount of toxic substances which apparently originated from contaminants introduced during the process of concentration. It seems worth while to investigate whether the substances bear any relation to primary toxic effects and chills observed in human beings under treatment with concentrated immune sera.

SUMMARY

Observations reported here seem to demonstrate the liberation or formation of a toxic principle in blood serum through a colloidal disturbance in the latter. The principle is able to elicit severe injury in tissues made vulnerable by a bacterial filtrate.

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BIBLIOGRAPHY

1. Shwartzman, G., *J. Exp. Med.*, 1930, **51**, 571.
2. Shwartzman, G., *Proc. Soc. Exp. Biol. and Med.*, 1929, **26**, 843.
3. Reiner, M., and Shwartzman, G., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 1063.
4. Shwartzman, G., *Proc. Soc. Exp. Biol. and Med.*, 1928, **26**, 207.
5. Shwartzman, G., *J. Exp. Med.*, 1930, **52**, 781.
6. Shwartzman, G., *J. Exp. Med.*, 1928, **48**, 247.
7. Sickles, G. U., *J. Immunol.*, 1931, **20**, 169.
8. Shwartzman, G., *J. Infect. Dis.*, 1931, **48**, 183.
9. Gratia, A., and Linz, R., *Compt. rend. Soc. biol.*, 1931, **108**, 238.
10. Klein, H. M., personal communication.
11. Debonera, G., Tzortzakis, N., and Falchetti, E., *Compt. rend. Soc. biol.*, 1932, **109**, 24.
12. Rous, P., Robertson, O. H., and Oliver, J., *J. Exp. Med.*, 1919, **29**, 283, 305. Rous, P., Wilson, G. W., and Oliver, J., *J. Exp. Med.*, 1920, **31**, 253.